Gillisia myxillae sp. nov., a novel member of the family *Flavobacteriaceae*, isolated from the marine sponge *Myxilla incrustans*

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A yellow-pigmented, Gram-negative, rod-shaped, strictly aerobic bacterium (strain UST050418-085^T) was isolated from the surface of a marine sponge, *Myxilla incrustans*, at Friday Harbor, WA, USA. The DNA G+C content of this strain was 34·6 mol%. The predominant fatty acids were i15 : 0, a15 : 0, i15 : 1, i16 : 0, i17 : 0 3-OH, 17 : 0 2-OH and summed feature 3, comprising i15 : 0 2-OH and/or 16 : 1ω7*c* (altogether representing 69·0 % of the total fatty acids). MK-6 was the only respiratory quinone detected. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the closest relatives of UST050418-085^T were members of the genus *Gillisia*, with sequence similarities of 93·2–96·6 %. Strain UST050418-085^T differed from its closest relatives by 11 to 18 phenotypic traits. Molecular evidence and phenotypic characteristics suggest that strain UST050418-085^T represents a novel species within the genus *Gillisia*. The name *Gillisia myxillae* sp. nov. is proposed, with UST050418-085^T (=JCM 13546^T=NRRL B-41416^T) as the type strain.

The family *Flavobacteriaceae* contains many marine species that form a well-defined 'marine clade' in phylogenetic trees based on 16S rRNA gene sequences (Bowman, 2004). *Gillisia* is a recently established genus within this family (Van Trappen *et al.*, 2004) and forms a phylogenetic cluster with the genera *Mesonia*, *Salegentibacter* and *Psychroflexus* (Nedashkovskaya *et al.*, 2005). Currently, there are five species in the genus *Gillisia*, all of which were isolated from marine environments, including a microbial mat and sea-ice algae in Antarctica, as well as seawater in the Sea of Japan (Van Trappen *et al.*, 2004; Bowman & Nichols, 2005; Nedashkovskaya *et al.*, 2005). In the present study, a novel member of the genus *Gillisia*, isolated from the surface of a marine sponge, is described.

During the characterization of bacteria isolated from the surface of the sponge *Myxilla incrustans* collected from Friday Harbor, San Juan Island, WA, USA, in April 2005, strain UST050418-085^T was isolated on an agar medium consisting of 3 g yeast extract l^{-1}, 5 g peptone l^{-1} and 0·22-μm-filtered seawater (referred to as marine agar hereafter) after 48 h incubation at 24 °C. Unless otherwise specified, all characteristics described are those of cultures grown on marine agar under these conditions. UST050418-085^T appeared as yellow, convex, circular colonies (1–2 mm in diameter) with an entire edge and a smooth surface. No diffusible pigment was observed.

The nearly complete 16S rRNA gene sequence of UST050418-085^T (1411 bp) was obtained bidirectionally with three replications as described by Lau *et al.* (2004). Phylogenetic analysis based on this nearly complete 16S rRNA gene sequence revealed that strain UST050418-085^T was a member of the family *Flavobacteriaceae*. Its closest relatives were *Gillisia mitskevichiae* KMM 6034^T (Nedashkovskaya *et al.*, 2005), *Gillisia limnaea* LMG 21470^T (Van Trappen *et al.*, 2004) and *Gillisia hiemivivida* IC154^T (Bowman & Nichols, 2005), with 96·6, 96·1 and 95·8 % 16S rRNA gene sequence similarity, respectively. A neighbour-joining (NJ) phylogenetic tree (Fig. 1) constructed using the ARB software package (Ludwig *et al.*, 2004) placed strain

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain UST050418-085^T is DQ202393.

A scanning electron micrograph of a cell of strain UST050418-085^T is available as supplementary material in IJSEM Online.

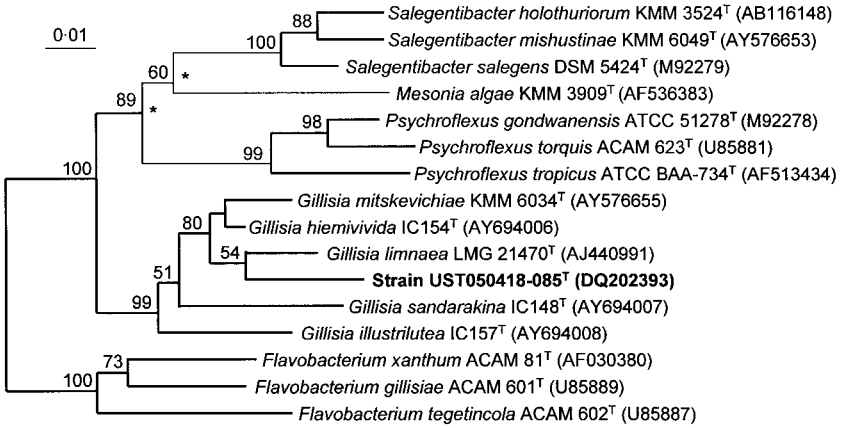

Fig. 1. Neighbour-joining tree showing the estimated phylogenetic relationships between UST050418-085^T and related species on the basis of 16S rRNA gene sequences. Strains belonging to the genus *Flavobacterium* were chosen as the outgroups. Asterisks indicate nodes that are also found in the maximum-parsimony tree. Thick lines indicate branches found in both the maximum-likelihood and maximum-parsimony trees. Bootstrap values over 50 % (500 replicates) are shown at nodes. GenBank accession numbers are shown in parentheses. Bar, 1 substitution per 100 nt.

UST050418-085^T within the cluster comprising the five species of the genus *Gillisia* and showed that the most closely related strain was *G. limnaea* LMG 21470^T. Sequence similarity shows the similarity between two sequences based on a comparison of their nucleotide sequences in overlapping regions nucleotide-by-nucleotide. However, construction of NJ trees uses a phenetic method, is distance-based using a clustering algorithm and takes other factors (e.g. type of mismatch, mutation, gap, etc.) into account to generate a similarity matrix using ARB software (Ludwig *et al.*, 2004). Therefore, the closest phylogenetic neighbour observed in the NJ tree is not necessarily the one with the highest sequence similarity because these comparisons are based on two different methods of calculating similarity. In fact, this phenomenon is common and found in other recent publications in the same genus or family (Van Trappen *et al.*, 2004; Lau *et al.*, 2005). Two other trees based on cladistic methods (i.e. character-based) were also constructed. One was made using the maximum-likelihood method, which selects the tree that is most likely to have produced the observed data; the other one was based on the maximum-parsimony method, which selects the tree that requires fewer evolutionary changes (Ludwig *et al.*, 2004). Both trees showed essentially the same topography for all species of the genus *Gillisia* (not shown). These results suggest that UST050418-085^T represents a novel species within the genus *Gillisia*.

The DNA G+C content of UST050418-085^T, as determined by HPLC according to Mesbah *et al.* (1989), was 34·6± 0·1 mol% (three replicates). This value is within the range of G+C contents observed for members of the genus *Gillisia* (i.e. 32·0–37·8 mol%). The cellular fatty acids of UST050418-085^T were determined using the Sherlock Microbial Identification System (MIDI) according to the manufacturer's protocol. The dominant fatty acids were i15 : 0, a15 : 0, i15 : 1, i16 : 0, i17 : 0 3-OH, 17 : 0 2-OH and summed feature 3, comprising i15 : 0 2-OH and/or 16 : 1ω7*c*, which altogether represent 69·0 % of the total fatty acids (Table 1). This fatty acid profile is similar to those of *G. limnaea* LMG 21470^T and *G. mitskevichiae* KMM 6034^T, confirming the close phylogenetic relationships observed for these bacteria (Fig. 1). MK-6 was the only respiratory quinone detected in UST050418-085^T, as determined using an HPLC method according to Collins (1994). Menaquinones extracted from *Cellulophaga lytica* (Nakagawa & Yamasato, 1993) and *Pedobacter heparinus* (Steyn *et al.*, 1998) served as references for MK-6 and MK-7, respectively.

Anaerobic growth was examined using the Oxoid Anaerobic System. Growth at different temperatures (4, 12, 20, 28, 36, 44 and 52 °C) and pH values (5, 6, 7, 8, 9 and 10) was monitored on marine agar for up to 10 days of incubation. The requirement for NaCl for growth was tested on a 1 % agar medium containing 5 g $MgCl_2$, 2 g $MgSO_4$, 0·5 g $CaCl_2$, 1 g KCl, 5 g peptone and different amounts of NaCl, adjusted to pH 7·5 using KOH (Isnansetyo & Kamei, 2003). Colony and cell morphology were examined using light ($\times$40 magnification) and scanning electron microscopy (6700F; JEOL) according to Neu *et al.* (2001) (see Supplementary Fig. S1 available in IJSEM Online). The reaction to Gram stain was determined using light microscopy according to Smibert & Krieg (1994). Gliding motility was observed under a phase-contrast light microscope (Olympia) after growth of the strain on quarter-strength marine 2216 medium (Oxoid) solidified with 1 % agar according to Bowman (2000). Susceptibility to antibiotics was tested according to Acar (1980). Flexirubin-type pigment production and carboxymethylcellulose hydrolysis were determined according to Bowman (2000). Casein hydrolysis was determined according to the procedures described by Norris *et al.* (1985); hydrolysis of Tweens 20, 40 and 80 and chitin was determined as described by Baumann & Baumann (1981), whereas hydrolysis of agar, DNA and starch was tested according to Smibert & Krieg (1994). Oxidase and catalase activities were determined as described by Smibert & Krieg (1994). Other enzyme activities, substrate utilization patterns, nitrate reduction and production of H_2S, indole and acetoin were tested using the commercial systems API 20E, API 20NE, API 50CH and

Table 1. Cellular fatty acid profiles of UST050418-085^T and other members of the genus *Gillisia*

Strains: 1, UST050418-085^T; 2, *Gillisia limnaea* LMG 21470^T; 3, *Gillisia mitskevichiae* KMM 6034^T; 4, *Gillisia hiemivivida* IC154^T; 5, *Gillisia sandarakina* IC148^T; 6, *Gillisia illustrilutea* IC157^T. Values given are mean percentages of the total fatty acid content. Data for reference strains were taken from Van Trappen *et al.* (2004), Bowman & Nichols (2005) and Nedashkovskaya *et al.* (2005). br, Branching position has not been determined; i, iso-branched fatty acids; a, anteiso-branched fatty acids; −, not detected. Summed feature 3 comprises i15 : 0 2-OH and/or 16 : 1ω7*c*.

Fatty acid	1	2	3	4	5	6
Straight-chain saturated						
15 : 0	−	−	4·4	4·3	4·3	4·3
16 : 0	1·6	0·3	−	1·8	0·5	1·7
15 : 0 2-OH	3·8	4·0	2·4	−	−	−
15 : 0 3-OH	−	−	−	1·1	0·7	0
16 : 0 3-OH	0·5	0·1	1·2	−	−	−
17 : 0 2-OH	7·3	13·6	3·8	−	−	−
17 : 0 3-OH	−	−	−	1·9	5·5	6·0
Straight-chain unsaturated						
15 : 1ω6*c*	0·3	1·2	1·9	2·3	1·7	1·4
17 : 1ω6*c*	2·5	1·9	4·7	−	−	−
Branched saturated						
i14 : 0	−	0·2	1·2	1·3	0·6	0·6
i15 : 0	16·6	7·2	7·5	3·5	3·2	3·2
a15 : 0	12·5	9·7	5·1	19·5	9·1	10·2
i16 : 0	6·2	7·4	9·3	2·7	15·8	14·4
a17 : 0	0·3	0·2	−	−	−	−
i15 : 0 3-OH	1·9	0·7	1·1	3·8	1·5	1·4
a15 : 0 3-OH	−	−	−	14·6	8·6	9·9
i16 : 0 3-OH	4·9	4·3	7·2	8·9	7·0	4·9
i17 : 0 3-OH	9·4	9·8	6·7	1·4	1·7	1·4
a17 : 0 3-OH	−	−	−	0·3	1·2	1·6
Branched unsaturated						
i15 : 1	8·9	9·2	11·8	3·0	13·2	14·6
a15 : 1	2·3	2·5	2·4	13·1	12·9	17·6
br16 : 1	−	3·0	6·0	4·2	6·2	3·8
i17 : 1	3·6	7·3	4·0	1·8	3·4	2·2
a17 : 1	3·5	7·7	2·1	4·7	3·0	3·3
Summed feature 3	7·7	8·4	11·1	4·0	4·4	2·8
Unknown	5·4	0·7	6·7	−	−	−

API ZYM (bioMérieux) and MicroLog 3 (Biolog). Cells for inoculating the API test systems were suspended in a sterile solution of seawater mixture at 22 ‰ salinity (MacDonell *et al.*, 1982). Growth on glycerol, D-glucose, sucrose, D-mannitol, D-galactose, starch, D-sorbitol, D-arabinose and D-melibiose as sole carbon sources was also tested using a medium containing 0·2 g $NaNO_3$, 0·2 g NH_4Cl, 0·05 g yeast extract and 4 % (w/v) carbon source in 1 l solution of seawater mixture at 35 ‰ salinity (Nedashkovskaya *et al.*, 2003).

The morphological, physiological and biochemical characteristics of UST050418-085^T are listed in the species description. Strain UST050418-085^T differed from its three closest relatives by 11 to 18 traits, indicated in Table 2. Molecular evidence, together with phenotypic characteristics presented in this study, suggest that UST050418-085^T constitutes a novel species within the genus *Gillisia*. The name *Gillisia myxillae* sp. nov. is proposed.

Description of *Gillisia myxillae* sp. nov.

Gillisia myxillae (my.xil′lae. N.L. fem. n. *Myxilla* systematic name of a genus of sponges; N.L. gen. fem. n. *myxillae* from *Myxilla*, referring to the isolation of the type strain from the sponge *Myxilla incrustans*).

Cells are Gram-negative, short rods (1·3–2·0 μm in length and 0·5 μm in width), strictly aerobic, devoid of gliding and flagellar motility. Upon cultivation on marine agar, colonies are yellow in colour, 2–4 mm in diameter, circular and convex with a smooth surface and an entire edge. Does not produce flexirubin-type or diffusible pigments. MK-6 is the only respiratory quinone. Growth occurs between pH 5·0 and 10·0 (pH 7·0–9·0 optimum) and between 4·0 and 28·0 °C (12·0–20·0 °C optimum), but not at 36·0 °C or higher. Requires NaCl (2·0–10·0 %; 4·0–6·0 % optimum) for growth. Predominant fatty acids are i15 : 0, a15 : 0, i15 : 1, i16 : 0, i17 : 0 3-OH, 17 : 0 2-OH and summed feature 3 (comprising i15 : 0 2-OH and/or 16 : 1ω7*c*), together representing 69·0 % of the total. Susceptible to benzylpenicillin (1·0 μg), chloramphenicol (1·0 μg), ampicillin (1·0 μg) and tetracycline (10·0 μg), but resistant to streptomycin and kanamycin (tested up to 100·0 μg). Acetoin, indole and H_2S are not produced. Nitrate is not reduced. Citrate is not utilized. Casein, starch, and Tweens 20 and 40 are degraded, but agar, Tween 80, chitin and carboxymethylcellulose are not. Positive for oxidase, DNase, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and tryptophan deaminase activities. Negative for gelatinase, urease, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase activities. Utilization of glycerol, D-glucose, sucrose, D-mannitol, D-galactose, starch, D-sorbitol, D-arabinose and D-melibiose as sole carbon sources is observed on agar medium supplemented with 4 % (w/v) carbon source and utilization of D-galacturonic acid, L-proline and putrescine is observed in the MicroLog 3 system. However, none of the carbon sources included in the API 20NE and 50CH systems are utilized. Acid is produced from L-arabinose, D-fructose, D-mannose, D-maltose, starch and glycogen in the API 50CH system, but no acid production is observed from the carbon sources in the API 20E system.

The type strain is UST050418-085^T (= JCM 13546^T = NRRL B-41416^T), isolated from the surface of the marine sponge

Table 2. Phenotypic characteristics that differentiate UST050418-085^T from the three most closely related members of the genus *Gillisia*

Strains: 1, UST050418-085^T; 2, *G. limnaea* LMG 21470^T; 3, *G. mitskevichiae* KMM 6034^T; 4, *G. hiemivivida* IC154^T. Data for reference strains were taken from Van Trappen *et al.* (2004), Bowman & Nichols (2005) and Nedashkovskaya *et al.* (2005). All strains are non-motile by gliding and incapable of hydrolysing agar, chitin and carboxymethylcellulose. All strains are positive for catalase and alkaline phosphatase activities. All strains are negative for reduction of nitrate, production of flexirubin-type pigments, H_2S, indole and acetoin, utilization of citrate, D-melibiose and L-rhamnose and lysine decarboxylase and ornithine decarboxylase activities. Y, Yellow; O, orange; +, positive; (+), weakly positive; −, negative; ND, not described.

Characteristic	1	2	3	4
DNA G+C content (mol%)	34·6	37·8	36·4	34·0
Pigment of cell biomass	Y	Y	Y	O
Na^+ requirement for growth	+	−	+	+
Tolerance of NaCl at:				
2·0 M	+	−	+	−
2·5 M	−	−	+	−
Growth temperature (°C)	4·0–28·0	5·0–30·0	4·0–31·0	−2·0 to 25·0
Susceptibility to:				
Tetracycline	+	−	+	ND
Streptomycin	−	−	+	ND
Hydrolysis of:				
Casein	+	−	+	−
Gelatin	−	+	+	+
Starch	+	−	−	+
DNA	+	−	+	−
Tween 20	+	+	−	ND
Tween 40	+	−	+	ND
Tween 80	−	−	+	+
Production of:				
Lipase (C14)	+	−	ND	ND
Urease	−	−	+	+
Tryptophan deaminase	+	−	−	−
Trypsin	−	+	ND	ND
β-Glucuronidase	−	(+)	−	−
α-Glucosidase	−	+	ND	+
β-Glucosidase	−	+	ND	+
Utilization of:				
D-Glucose	−	+	+	+
Sucrose	−	−	+	+
L-Arabinose	−	−	−	+
D-Mannose	−	−	−	+
N-Acetylglucosamine	−	−	+	+
D-Maltose	−	−	ND	+
Propionate	−	+	ND	+

Myxilla incrustans at Friday Harbor, San Juan Island, WA, USA. The DNA G+C content of strain UST050418-085^T is 34·6 mol%.

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References

Acar, J. F. (1980). The disk susceptibility test. In *Antibiotics in Laboratory and Medicine*, pp. 24–54. Edited by V. Lorian. Baltimore: Williams & Wilkins.

Baumann, P. & Baumann, L. (1981). The marine gram-negative eubacteria: genera *Photobacterium*, *Beneckea*, *Alteromonas*, *Pseudomonas* and *Alcaligenes*. In *The Prokaryotes*, vol. 1, pp. 1302–1331. Edited by M. P. Starr, H. Stolp, H. G. Trüper, A. Balows & H. Schlegel. Berlin: Springer.

Bowman, J. P. (2000). Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of

Cytophaga uliginosa (ZoBell and Upham 1944) Reichenbach 1989 as *Cellulophaga uliginosa* comb. nov. *Int J Syst Evol Microbiol* **50**, 1861–1868.

Bowman, J. P. (2004). Psychrophilic prokaryote structural-functional relationships, biogeography and evolution within marine sediment. *Cell Mol Biol* **50**, 503–515.

Bowman, J. P. & Nichols, D. S. (2005). Novel members of the family *Flavobacteriaceae* from Antarctic maritime habitats including *Subsaximicrobium wynnwilliamsii* gen. nov., sp. nov., *Subsaximicrobium saxinquilinus* sp. nov., *Subsaxibacter broadyi* gen. nov., sp. nov., *Lacinutrix copepodicola* gen. nov., sp. nov., and novel species of the genera *Bizionia*, *Gelidibacter* and *Gillisia*. *Int J Syst Evol Microbiol* **55**, 1471–1486.

Collins, M. D. (1994). Isoprenoid quinones. In *Chemical Methods in Prokaryotic Systematics*, pp. 265–310. Edited by M. Goodfellow & A. G. O'Donnell. Chichester: Wiley.

Isnansetyo, A. & Kamei, Y. (2003). *Pseudoalteromonas phenolica* sp. nov., a novel marine bacterium that produces phenolic anti-methicillin-resistant *Staphylococcus aureus* substances. *Int J Syst Evol Microbiol* **53**, 583–588.

Lau, S. C. K., Tsoi, M. M. Y., Li, X., Plakhotnikova, I., Wu, M., Wong, P. K. & Qian, P. Y. (2004). *Loktanella hongkongensis* sp. nov., a novel member of the α-*Proteobacteria* originating from marine biofilms in Hong Kong waters. *Int J Syst Evol Microbiol* **54**, 2281–2284.

Lau, S. C. K., Tsoi, M. M. Y., Li, X. & 7 other authors (2005). *Winogradskyella poriferorum* sp. nov., a novel member of the family *Flavobacteriaceae* isolated from a sponge in the Bahamas. *Int J Syst Evol Microbiol* **55**, 1589–1592.

Ludwig, W., Strunk, O., Westram, R. & 29 other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.

MacDonell, M. T., Singleton, F. L. & Hood, M. A. (1982). Diluent composition for use of API 20E in characterizing marine and estuarine bacteria. *Appl Environ Microbiol* **44**, 423–427.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Nakagawa, Y. & Yamasato, K. (1993). Phylogenetic diversity of the genus *Cytophaga* revealed by 16S rRNA sequencing and menaquinone analysis. *J Gen Microbiol* **139**, 1155–1161.

Nedashkovskaya, O. I., Kim, S. B., Hans, S. K. & 7 other authors (2003). *Mesonia algae* gen. nov., sp. nov., a novel marine bacterium of the family *Flavobacteriaceae* isolated from the green alga *Acrosiphonia sonderi* (Kütz) Kornm. *Int J Syst Evol Microbiol* **53**, 1967–1971.

Nedashkovskaya, O. I., Kim, S. B., Lee, K. H., Mikhailov, V. V. & Bae, K. S. (2005). *Gillisia mitskevichiae* sp. nov., a novel bacterium of the family *Flavobacteriaceae*, isolated from sea water. *Int J Syst Evol Microbiol* **55**, 321–323.

Neu, B., Voigt, A., Mitlohner, R. & 7 other authors (2001). Biological cells as templates for hollow microcapsules. *J Microencapsul* **18**, 385–395.

Norris, J. R., Ribbons, D. W. & Varma, A. K. (editors) (1985). *Methods in Microbiology*, vol. 18. London: Academic Press.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characteristics. In *Methods for General and Molecular Biology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Steyn, P. L., Segers, P., Vancanneyt, M., Sandra, P., Kersters, K. & Joubert, J. J. (1998). Classification of heparinolytic bacteria into a new genus, *Pedobacter*, comprising four species: *Pedobacter heparinus* comb. nov., *Pedobacter piscium* comb. nov., *Pedobacter africanus* sp. nov. and *Pedobacter saltans* sp. nov. Proposal of the family *Sphingobacteriaceae*. *Int J Syst Bacteriol* **48**, 165–177.

Van Trappen, S., Vandecandelaere, I., Mergaert, J. & Swings, J. (2004). *Gillisia limnaea* gen. nov., sp. nov., a new member of the family *Flavobacteriaceae* isolated from a microbial mat in Lake Fryxell, Antarctica. *Int J Syst Evol Microbiol* **54**, 445–448.